Zinc biofortification through biofertilizers, foliar and soil application in maize (*Zea mays* L.)

ABSTRACT

Aims: Here is clearly aim of study is to achieve the maximum grain yield and for improve the economic conditions of farmers.

Study design: The experiment was conducted in a split-plot design.

Place and duration of study: The field experiment was conducted to study the effect of biofertilizers, foliar and soil application of zinc fertilizers in maize (*Zea mays* L.) at the research farm of the Khalsa College, Amritsar, Punjab (India) (31°63'N latitude, 74°83' E longitude and 234 m above sea level) during rainy seasons of 2018 and 2019.

Methodology: The four levels of biofertilizers and foliar application as main plot [recommended dose of fertilizer (RDF: 150 kg nitrogen: 27.3 kg phosphorus: 25 kg K ha^{-1}), RDF + Zn solubilizing bacteria (ZnSB), RDF + ZnSB + vesicular-arbuscular mycorrhiza (VAM) + 0.5% foliar $ZnSO_4.7H_2O$) at silking and early milk stage] and four subplot treatment of soil application of $ZnSO_4.7H_2O$ @ 12, 25 and 50 kg/ha at the time of sowing along with no soil Zn control were evaluated in three replications.

Results: Foliar application of zinc in addition to biofertilizers (ZnSB + VAM) produced significantly higher growth, yield and quality over control treatment. Soil application of zinc sulphate (50 kg ha^{-1}) significantly improved growth, grain yield and Zn harvest index, Zn mobilization efficiency index and recovery efficiency over no Zn control treatment. The grain zinc content, zinc uptake and zinc use efficiencies were significantly maximum with the application of VAM + ZnSB along with 0.5% zinc sulphate foliar at silking and early milk stage than ZnSB, VAM and control treatment.

Conclusion: Zn application of soil and foliar improved the growth, yield and quality parameters of maize.

Keywords: Maize, biofortification, biofertilizers,zinc application

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1. INTRODUCTION

"Zinc (Zn) deficiency is a well-recognized problem occurring in both plants and human beings.Imbalanced and indiscriminate application of high analysis fertilizers to different crops with low or no use of organic manures leads to deficiency of micronutrients. Around 50% of Indian soils are deficient in Zn and it is the fourth most important yield-limiting nutrient after nitrogen, phosphorus and potassium" (Das and Green 2013). Nowadays, in humans, Zn deficiency has become a serious problem affecting nearly half of the world's population (Cakmak, 2008) and causing impairment in brain development, skin lesions, anorexia, immune suppression, loss of taste and weakness in body muscles (Black et al. 2009). "The problem of zinc deficiency is more serious in young children as it causes diarrhoea, pneumonia, childhood dwarfism, morbidity and mortality under-five years of age" (Cakmak and Kutman2018 and Graham 2007). "Because of the emergence of Zn as an important nutritional problem, especially in developing countries, the need for biofortification of plants with higher Zn contents in grains" was suggested by Bouis et al. (2011). "Biofortification of crops can be attained byplant breeding and genetic engineering and fertilization"(Sadeghzadeh 2013). "Agronomic biofortification (fertilizer approach) appears to be important in keepingan adequatequantity of available Zn in soil solution and increases its transport to the seeds during the reproductive growth stage through foliar application and is the safe and cheaper method" (Ram et al.2015).

"Maize (*Zea mays* L.) is a dietary staple food crop that ranks third-largest cereal crop after wheat and rice with a production of 27.80 million metric tonnes during 2018-2019"(Anonymous 2019)."The application of Zn is reported to enhance maize grain yield around the world" (Harris et al. 2007). "But Zn biofortification of maize grain has not received much attention, although maize is an important source of carb-rich diet in developing countries, where a major portion of the population is vegetarian" (Prasad 2010). "Animal-based foods have a very minute quantity of Zn as compared to vegetarian foods"(Cakmak and Kutman 2018)."Among the cereals considered, wheat was by far the most responsive to foliar Zn spray about increases in grain Zn followed by rice"(Ram et al. 2015;Zhou et al. 2012 and Phattarakul et al. 2012). "Maizeappeared to be less responsive to foliar Zn fertilization"(Mabesa et al. 2013). "Soil Zn is converted into various unavailable forms that depend upon soil type and chemical reactions and unavailable zinc compounds can be reversed to available form through bioaugmentation of Zn solubilizing bacteria" (Hussain et al. 2015). "Similarly, the application of VAM biofertilizer results in enhanced availability and uptake of Zn by acidifying the rhizosphere and provides the ability for the host plant to utilize the tightly bound Zn by the external mycelium" (Subramanian et al. 2009)."Zn is essential to all forms of life because of its vital role in gene expression, cell development and replication" (Hambridge 2000).Keeping in view the significance of Zn in mitigating malnutritionand increasing the yield and quality of maize, the present investigations were undertaken to study the effect of integrated use of biofertilizers, foliar application and soil application on grain yield and grain Zn of maize.

2.MATERIAL AND METHODS

2.1.Location and soil characteristics

The field experiment was carried out during the rainy seasons of 2018 and 2019 at the Khalsa College, Amritsar, Punjab, India (31^{o}63'N latitude & 74^{o}83' E longitude and about 234 m above sea level). The soil of the experimental field was sandy loam in texture with normal pH (7.7) and electrical conductivity (0.40mmhos cm^{-1}). The soil rated medium in organic carbon (0.46), low in available nitrogen (168 kg ha^{-1}), medium in available phosphorus (28.5 kg ha^{-1}), medium in available potassium (330 kg ha^{-1}) and low in available Zinc (0.53 ppm).

2.2.Experimental treatments and design

The experiment was conducted in a split-plot design with four main plot treatments as biofertilizers and foliar [recommended dose of fertilizer (RDF: 150 kg nitrogen: 27.3 kg phosphorus: 25 kg K ha^{-1}), RDF + Zn solubilizing bacteria (ZnSB), RDF + ZnSB + Vesicular-arbuscular mycorrhiza (VAM)+ 0.5% foliar $ZnSO_4.7H_2O$) at silking and early milk stage] and four subplot treatment of soil application of $ZnSO_4.7H_2O$ @ 12, 25 and 50 kg/ha at the time of sowing with three replications.The PMH-7 variety and Glomus VAM species used in experiment according to treatments given by different researchers. The sowing was done by dibbling two seeds per hill keeping row to row spacing of 60cm and plant to plant spacing of 20cm on a well-prepared seedbed.Nitrogen was applied in the form of urea in three splits: one-third nitrogenwas applied at sowing, the other one third at a knee-high stage near the plant base and the remainingone-third nitrogen at the pre-tasselling stage. Phosphorus and potassiumfertilizers wereapplied as basal doses to the crop.

2.3.Datacollection and analysis

The leaf area index and dry matter accumulation of randomly selected five plants were measured at 70 days after sowing. Randomly selectedfive plants from each plot at harvest stage tomeasure various parameterslike plant height, cob length, grain weight per cob, 100-grain weight. The Zn concentration in grains and stover was calculated by MPAES-Microwave plasma atomic emission spectroscopy after wet digestion of grains. The grain samples were digested in a di-acid mixture of nitric acid (HNO_3) and perchloricacid ($HClO_4$) in the ratio of 3:1 for the analysis of Zn. The Zn uptake of maize grains and stover was computed by multiplying Zn concentrations (ppm) with corresponding yield (t ha^{-1}) and was expressed in g ha^{-1}.

The Zn harvest index (ZnHI)and Zn mobilization efficiency index (ZnMEI)were calculatedemployingexpressions as proposed by Shivay and Parsad (2014):

$$\mathrm{ZnHI} = \frac{\mathrm{Zns}}{\mathrm{Znt}} \times 100 \quad (1)$$

$$\mathrm{ZnMEI} = [\text{Grain Zn concentration} \div \text{Straw Zn concentration}] \quad (2)$$

Zn_s = Zn uptake by grain at harvest, and Zn_t = Zn uptake by whole crop (grain + stover) at harvest.

The economics was calculated based on the prevailing prices of inputs and outputs and expressed as US$ per ha.The analyses of variances were carried out on pooled data. All the data were statistically analyzed by analysis of variance using split-plot design in CPCS-I software.

3.RESULTS

3.1.Growth, yield attributes and yields of maize

The application of ZnSB + VAM + 0.5% foliar Zn hada significanteffect on plant height, leaf area index and dry matter accumulation at silking stage over the control treatment (Table 1). The tallest plants were recorded in ZnSB + VAM + 0.5% foliar Zn which was similar to VAM and ZnSB but significantly higher than no biofertilizer control. However, ZnSB alone was not able to increase plant height as compared to no biofertilizer control All the zinc fertilization treatments i.e. Zn_{12}, Zn_{25} and Zn_{50} significantly increased the plant height as compared to no Zn control treatment. The dry matter accumulation recorded in ZnSB + VAM + 0.5% foliar Zn (219.8 g/plant) was significantly higher than no biofertilizer control and ZnSB but statistically at par with the VAM treatments. The dry matter accumulated in Zn50 was found to be the highest (221.7 g/plant) which was significantly higher than rest of the Zn treatments and no zinc control plot. The dry matter accumulated in $Zn_{12}$and Zn_{25} was statistically similar to each other but significantly higher than no Zn control treatment. The LAI ranged from 3.78 in no biofertilizer control treatment to 4.41 in RDF + ZnSB + VAM + 0.5% foliar Zn. The highest LAI recorded in RDF + ZnSB + VAM + 0.5% foliar Zn was statistically similar to ZnSB and VAM but significantly higher than no biofertilizer control plot. As recorded in plant height, all zinc fertilization treatments (Zn_{12}, Zn_{25} and Zn_{50}) recorded similar LAI and significantly higher than no Zn control plot. The response of all the growth characters in relation to zinc fertilization and biofertilzation was not significant.

The yield attributes and grain/straw yield were significantly influenced by main and sub plot treatments (Table 2). The highest cob length was recorded in

ZnSB+VAM+ZnFoliar which was statistically at par with VAM and ZnSB but significantly higher than no biofertilizer control. The cob length recorded in all the Zn treatments was significantly higher than recorded in no Zn control treatment.The grain per cob recorded in ZnSB+VAM+Zn was the highest which was significantly higher than all other treatments. The grains per cob recorded in VAM and ZnSB treatments were statistically similar but significantly higher than no Zn control treatment. The Zn_{50} treatment recorded the highest grains per cob which were significantly higher than recorded in all other Zn treatments and no Zn control. The minimum grains per cob were recorded in no Zn treatment. The 100-grain weight was not significantly influenced by biofertilizers and foliar Zn fertilizer application treatments.

The highest grain yield of maize was recorded in ZnSB + VAM + 0.5% foliar Zn (5.61 t ha^{-1}) which was 7.88% and significantly higher than no biofertilizer control. Applications of ZnSB and VAM were also able to enhance grain yield over the control but the increase was only 5.2 and 7.1% over the control respectively. But the grain yield recorded in ZnSB, VAM +ZnSB + VAM + 0.5% foliar Zn was statistically similar. All Zn fertilization treatments recorded significantly higher grain yield than no Zn fertilizer treatment. TheZn fertilization treatment Zn_{50} recorded the highest grain yield which was 10.7% higher than no Zn control plot. The grain yield recorded in Zn_{25} was similar to Zn_{50} statistically. Although, the grain yield recorded in Zn_{12} was significantly higher than no Zn fertilizer but the increase was only 5.44%.

The stover yield recorded in all the biofertilizer treatments was similar with each other but significantly higher than recorded in no biofertilizer control plot. The stover yield increase recorded in ZnSB + VAM + 0.5% foliar Zn was 12.3% higher than no biofertilizer control plot. Among Zn fertilization treatments, Zn_{50} recorded 9.7% higher stover yield than no Zn control plot.

Table 1: Effect of Zn biofertilizers, foliar and soil application on growth parameters of maize (*Zea mays* L.)

Treatments	Plant height (cm)	Leaf area Index	Dry matter production (g/plant)
Biofertilizers and foliar			
Control (Recommended dose of NPK)	195.50	3.78	207.01
Zinc solubilising bacteria (ZSB) at sowing	199.74	4.21	214.81
Vesicular Arbuscular Mycorrhiza (VAM) at sowing	200.72	4.20	216.85

ZSB+VAM at sowing with 0.5% zinc sulphate foliar application at 45 and 60 DAS	202.07	4.41	219.75
CD (p=0.05)	4.17	0.21	5.25
Soil application			
Control(Recommended dose of NPK)	195.3	3.76	208.21
Zinc sulphate @12 kg ha^{-1} at sowing	199.12	4.23	212.51
Zinc sulphate @25 kg ha^{-1} at sowing	201.44	4.29	215.99
Zinc sulphate @ 50 kg ha^{-1} at sowing	202.18	4.34	221.71
CD (p=0.05)	3.73	0.37	4.09
Interaction	NS	NS	NS

Table 2 Effect of Zn biofertilizers, foliar and soil application on yield and yield attributes of maize (*Zea mays* L.)

Treatments	Cob length (cm)	No. of grains per cob	Test weight (100 grains weight)	Grains yield (qha^{-1})	Stover yield (qha^{-1})
Biofertilizers and foliar					
Control (Recommended dose of NPK)	16.95	298.41	20.0	51.95	74.95
Zinc solubilising bacteria (ZSB) at sowing	18.62	315.91	20.80	54.68	80.01
Vesicular Arbuscular Mycorrhiza (VAM) at sowing	19.24	316.25	20.97	55.65	80.66
ZSB+VAM at sowing with 0.5%	19.82	338.00	21.07	56.10	84.18

zinc sulphate foliar application at 45 and 60 DAS					
CD (p=0.05)	1.39	12.54	NS	2.62	4.64
Soil application					
Control(Recommended dose of NPK)	17.48	299.50	20.55	51.41	75.35
Zinc sulphate @12 kg ha^{-1} at sowing	18.59	317.08	21.43	54.17	80.80
Zinc sulphate @25 kg ha^{-1} at sowing	19.14	323.67	20.49	55.94	81.07
Zinc sulphate @ 50 kg ha^{-1} at sowing	19.47	328.33	21.40	56.85	82.59
CD (p=0.05)	1.10	13.77	NS	2.76	5.14
Interaction	NS	NS	NS	NS	NS

3.2.Zn content in grain and uptake

The highest grain Zn, straw Zn and Zn uptake by grain and straw were recorded in ZnSB + VAM + 0.5% foliar Zntreatment.

The grain Zn recorded in ZnSB + VAM + 0.5% foliar Zn (40.8 mg kg^{-1}) was significantly higher than rest of the treatments which was 8.5 mg kg^{-1} and 26.3% higher than recorded in no biofertilizer control plot. The grain Zn concentration recorded in ZnSB and VAM was 9.6 and 15.25% high grain Zn than recorded in no biofertilizer control.In the soil Zn application treatments, the highest Zn content in grain (39.6 mg kg^{-1} was recorded in Zn_{50} which was similar to Zn_{25} but significantly higher than the rest of the treatments including no Zn control plot. The minimum Zn content in grain (32.1 mg kg^{-1}) was recorded in no Zn control plot.

The Zn content in stover was ranged from 43.0 mg kg^{-1} in no biofertilizer control plot to 53.1 mg kg^{-1}in ZnSB + VAM + 0.5% foliar Zn. The treatment ZnSB + VAM + 0.5% foliar Zn recorded significantly higher stover zinc than other treatments. The stover Zn recorded in ZnSB and VAMwas similar but both of the treatments recorded siginificantly higher stover Zn than no biofertilizer control.

The highest Zn uptake by grains was recorded in ZnSB + VAM + 0.5% foliar Zn (229.6 kg/ha) which was significantly higher than other treatments. The Zn uptake by grain in VAM was significantly higher than ZnSB. Similarly, grains Zn uptake was significantly higher in ZnSB than no biofertilizer control plot.The Zn uptake recorded in $Zn_{50}$was the highest which was statistically at par with Zn_{25}but significantly higher than no Zn control plot. The grain Zn uptake recorded in Zn_{25} and Zn_{12} was statistically similar to each other.

The Zn uptake in stover was the highest in ZnSB + VAM + 0.5% foliar Zn (444.5 g ha-1) which was significantly higher than all other treatments. The stover Zn uptake recorded in VAM and ZnSB was similar statistically but significantly higher than no biofertilizer treatment.Among Zn fertilization treatments, all higher Zn doses treatments recorded significantly higher stover Zn uptake than the lower dose of Zn treatment with the highest of 719.6 g ha^{-1} in Zn_{50}.

Table 3: Effect of Zn biofertilizers, foliar and soil application on quality attributes of maize (*Zea mays* L.)

Treatments	Zinc content in grains (mg kg^{-1})	Zn uptake by grains (g ha^{-1})	Zinc content in stover (mg kg^{-1})	Zn uptake by stover (g ha^{-1})
Biofertilizers and foliar				
Control (Recommended dose of NPK)	32.28	168.27	42.94	321.89
Zinc solubilising bacteria (ZSB) at sowing	35.35	193.31	47.70	379.79
Vesicular Arbuscular Mycorrhiza (VAM) at sowing	37.15	205.15	49.15	393.86
ZSB+VAM at sowing with 0.5% zinc sulphate foliar application at 45 and 60 DAS	40.78	229.63	53.07	444.45
CD (p=0.05)	2.92	11.87	2.77	16.14
Soil application				
Control (Recommended dose of NPK)	32.14	165.97	44.30	333.09

Zinc sulphate @12 kg ha^{-1} at sowing	36.49	198.30	48.02	387.09
Zinc sulphate @25 kg ha^{-1} at sowing	37.35	208.46	49.71	400.17
Zinc sulphate@ 50 kg ha^{-1} at sowing	39.58	216.31	50.84	407.15
CD(p=0.05)	3.10	18.96	3.13	20.13
Interaction	NS	NS	NS	NS

4.DISCUSSION

In India, maize is cultivatedon a wide range of environmental conditions, spreading from extreme semi-arid to sub-humid and humid regions. It is a chief source of calories and minerals for most countrysidepeople, farm animals and poultry birds. But unfortunately, Zn concentration in the grain is innately very low, mostly when cultivated on Zn-deficient soils. Continuoususe of inorganic zinc fertilizer partially provides the plant need as 96–99% of applied Zn is transformed into different insoluble forms depending upon the soil kinds and physicochemical reactions.

4.1.Biofertilizer and foliar Zn application

The application of ZnSB or VAM and together ZnSB + VAM + 0.5% foliar Zn fertilizer application gave better plant height and leaf area index (LAI) than no biofertilizer control plot. The better growth was due to more availability of Zn in ZnSB and VAM and foliar fertilization in 0.5% Zn foliar. The availability of Zn increases with the production of organic acids in the soil. Ramesh et al. (2014) stated that the formation of acidic conditions in liquid media and solubilization of zinc by rhizobacteria are due to the creation of different organic acids. Production of several organicacids by microorganisms has been reported to be themain cause of zinc solubilization (Seshadreet al.(2002), Fasimet al.(2002) and Hussain et al. (2015)].Improved growth with ZnSB inoculation might be due to the increase in the availability of nutrients (Burd et al. 2000). Saboor et al. (2021) observed that Arbuscular mycorrhizal fungi inoculation helped to improve photosynthesis, transpiration, and stomatal conductance which helped to improve the growth characters.

The higher yield attributes liker grains per earhead recorded in ZnSB or VAM or ZnSB + VAM + 0.5% foliar Zn fertilizer application.ZnSB and VAM facilitated the acidification of rhizosphere, solubilization of precipitated Zn compounds resulting in the improvement in the soil available Zn status leading to a higher amount of nutrient uptake by the crop, translocated from source to sink which had reflected in the grain yield. The combined application of arbuscular mycorrhizal fungi and zinc solubilizing bacteria had a significant influence over untreated control on maize growth and yield.

The biofertilizers and foliar Zn application improved leaf area index might be due to rapid absorption of zinc nutrient through leaf, roots and this culminates increase in cell division and elongation, chlorophyll content and photosynthesis. Zn application to foliage is easily absorbed through foliage and transported within the plants when they are dissolved in water and sprayed on them resulting in higher dry matter production (Ram et al. 2015).The grain Zn and stover Zn content were significantly higher with the application of ZnSB and VAM than recommended fertilizers.The timely availability of the Zn nutrient to the plant at the critical growth stages through foliar application and ZnSB + VAM inoculation enhances Zn supply to the maize plant by improving the available Zn enabling the plant to maintain higher nutritional status. The maximum increase was recorded with ZnSB + VAM + 0.5% Zn foliar application due to direct application of Zn on the crop foliage and translocation is quick. The increase in Zn content in grain was comparatively less as compared to wheat and rice. Ram et al. (2015) reported that the application of 0.5% Zn enhanced the grain Zn in rice and wheat by 35 and 100% over no Zn foliar control. Suganya and Saravanan (2016) alsostatedthat Zn uptake by maize grain was increased to 44.1% by the application of VAM + ZSB. The same trend was also observed for the uptake of Zn by maize stover. These results are supportedby the findings of Kanimozhi (2015) who reported the increased nutrient uptake due to the application of microbial inoculation to the crop. Better Zn mobilization in 0.5% Zn foliar application was due to better translocation from leaves to grains and its accumulation in maize grains.Kumar et al (2021) also reported that that foliar zinc application improves the leaf zinc and growth of leaves in fodder maize. Guletie at al. (2013) reported from short term pot culture experiment with maize that seed bacterization with P29 @ 10 $g \cdot kg^{-1}$ significantly enhanced total dry mass and uptake of N, K, Mnand Zn.

4.2.Soil Zn application

The zinc sulphate application in soil increased the growth and yield attributes of the maize with the highest in Zn_{50}. The higher growth and yield attributes in higher Zn dose were due to the supply of Zn nutrients to the plants which improved yield characters. As the result of yield attributes, the grain yield was also higher in Zn50 which was similar to Zn_{25} and Zn_{12}. Improvement in yield and yield attributes by soil application might be attributed to the channelization of photosynthates during the reproductive stage that has been influenced by zinc by way of its involvement in electron transport and activation of various enzymes (Paramasivan et al. 2011).The soil Zn application also enhanced the carbohydrates supply to kernels, increasing yield components like cob length, the number of grains cob^{-1} and test weight, which hasa direct influence on the grain yield.Ruffo, Olsonb and Daveredec(2016) reported better yield attributes and yield with the application of 11.21 kg Zn ha^{-1} in maize. Zn content and uptake were higher in maize plants might be due to the presence of zinc micronutrients in huge amounts in soil and the slow release of these micronutrients into the soil solution which facilitated more Zn uptake (Prasad 2010). Coccina et al. (2019) described that improved growth due to Zn application is associated with increased Zn uptake by the plant.

5. CONCLUSION

Zinc solubilizing bacteria and vesicular-arbuscular mycorrhiza application at sowingalong witha foliar application of zinc sulphate (0.5%) at silking and early milk stage attainedhigher growth, grain yield, Zn content in grains and better returns.Theapplication of zinc sulphate (12 kg/ha) in soil could be helpful for the improvementof growth, grain yield, quality parameters.Thus, foliar zinc application in addition to biofertilizers and soil applicationenhance the grain yield and grain Znwhich may contribute to human nutrition under low zinc soils.

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